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Adult Salinity Tolerance and Larval Salinity Requirement of the Freshwater Shrimp *Palaemon paucidens* in Japan

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Summary

Although the shrimp *Palaemon paucidens* lives in freshwater, somewhat saline water is generally required for successful development of early life history stages of this species. There are two genetically separated types (A- and B-types) of this species, the A-type appears in rivers, ponds and lakes, but the B-type only occurs in the downstream parts of rivers. The salinity tolerance of adults and the salinity requirement of larvae were compared between the two types.

Adults of the B-type had a higher survival rate than adults of the A-type when cultured at 30‰ for 20 days. The larvae of both types could survive and metamorphose to post larvae at 10‰ and 30‰. All larvae of the B-type younger than third instars died when kept in freshwater medium, but the larvae of the A-type could survive and metamorphose to the postlarvae in freshwater.

From the above results, the A-type appears to be adapted to freshwater better than the B-type. The A-type might have radiated to freshwater areas before the B-type.

Introduction

Palaemon paucidens is one of the most common freshwater shrimp; it inhabits rivers, lakes and ponds in Japan. Chow and Fujio (1) discovered that there are two genetically separated types (A- and B-types) based on electrophoretically detectable differences in isozymic allele frequencies. Both types occur in the same river system. Fidhiany *et al.* (2) found a completely divergent loci, *Sod*, in the two types, and suggested that the two types were divergent at the subspecies level. Mating experiments have indicated that the two types are isolated reproductively (3, 4). Although morphological differences have been also reported between the two types (5, 6), it is not easy to distinguish the two types based on morphology alone. We present data on the physiological, ecological and evolutionary differences between the two types related to their habitat differences.

As in the case of another freshwater shrimp, *Macrobrachium* nipponense (7, 8), a certain level of salinity is generally necessary for the early development of *Palaemon paucidens* (9). Mashiko (9) has suggested that *P. paucidens* has radiated from seawater and that differences in salinity tolerances and requirements might exist among localities or populations of this species in relationship to evolutionary processes.

Although during summer, when they are immature, the two types can be caught in the middle sections of the same river (1), the B-type adults occur mainly in downstream areas of rivers while the A-type occur mainly in the middle and upstream parts of rivers, and in lakes and ponds. Since their habitats differ, the salinity tolerances and requirements of the two types of *Palaemon paucidens* are expected to also differ.

The objectives of the present study are to estimate the salinity tolerance in adults and the salinity requirements in larvae of the A- and B-types of *P. paucidens.* Evolutionary radiation in the two types is discussed.

Materials and methods

Adult A-type specimens of *Palaemon paucidens* were collected from Hinata Pond and Kamitomita Pond which are located in Kannari Town, and from Marutazawa Pond and the Natori River which are located in Sendai City. Those of the adult B-type were collected from the Hirose River in Sendai City and the Kuji River in Mito City. Larvae of *Palaemon paucidens* were obtained from adult females collected from the same localities mentioned above, with the exception of the Kuji River B-type. The method used to rear the adults and to obtain larvae were identical to those in previous reports (3–5).

Classification of the two types was performed by comparison of electrophoretically detectable isozyme loci including the *Sod* locus as previously reported (2). After the salinity tolerance experiments, all of the adult individuals and females used for spawning were identified the type A or B based on electrophoresis. In the present study, the two types were never observed in a sample from one location, either A or B alone were observed.

An experiment on salinity tolerance in adults was conducted as follows : each adult was taken from the freshwater rearing aquarium, which had a temperature of 23°C, and put into a 3-liter aquarium filled with seawater with either a salinity of 23‰ or 30‰ and a water temperature of 23°C. The culture medium used in the present study was microfiltered natural seawater supplied from Tohoku National Fisheries Research Institute. Seawater with a salinity of 23‰ was obtained in September 1988 and seawater with a salinity of 30‰ was obtained in July 1988 and 1989. Salinity tolerance was measured by survival rate during 20 days rearing in each treatment.

The experiment on salinity requirements in larvae was conducted as follows :

Females were reared in 3-liter aquaria filled with freshwater at a temperature of 26°C. Larvae were obtained from each berried female. Thirty individual larvae from a female were taken out with a pipette and put into 500-ml beakers filled with medium with a salinity of 30‰, 10‰ or freshwater. The water temperature was kept at 26°C during the larval stage. About 2/3 of the cultured medium was changed every day. Nauplii of brine shrimp were provided as food. Brine shrimp nauplii were added once or twice a day in order to maintain adequate food levels in each beaker. The survival of the larvae was determined by counting the number of live and dead individuals every day until metamorphosis. This was done in order to avoid miscount caused by cannibalism. The survival rate (S) was calculated as follows:

$$S = 1 - [D/(D + AF)]$$

where D denotes the number of dead individuals and AF the number of live individuals. The final survival rate to metamorphosis was calculated with the above formula taking AF as the number of first instar postlarvae, just after metamorphosis from the larva.

Results

Salinity tolerance in adults: Table 1 shows the survival rates in adults reared for 20 days in experimental culture media with salinities of 23‰ and 30‰. All individuals of both A- and B-types survived well in the medium with the salinity

TABLE 1. *Survival of adult* Palaemon paucidens *cultured in medium with salinities of 23‰ and 30‰ for 20 days*

Type / Locality	Salinity level 23‰	30‰
A-type		
Hinata Pond	100% (3)	13.3% (30)
Kamitomita Pond	100% (3)	43.7% (16)
Marutazawa Pond	100% (4)	36.7% (30)
Natori River	100% (3)	33.3% (30)
mean	100%	31.3%
B-type		
Hirose River	100% (6)	70.0% (30)
Kuji River	100% (3)	90.0% (10)
mean	100%	80.0%

Number in parenthesis represent the number of individuals tested

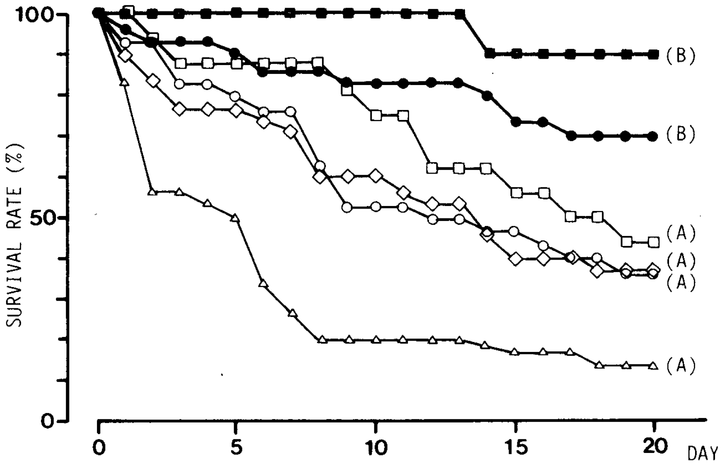

Fig. 1. Survival of adult *P. paucidens* cultured at a salinity of 30‰.
A-type : △ ; Hinta Pond, □ ; Kamitomita Pond, ◇ ; Marutazawa Pond, ○ ; Natori River.
B-type : ● ; Hirose River, ■ ; Kuji River.

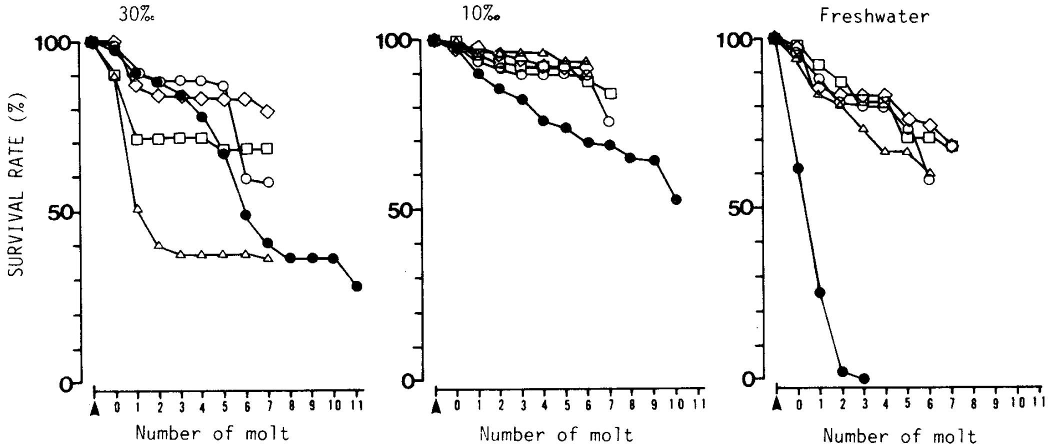

Fig. 2. Survival of larval instars of *P. paucidens* cultured at salinities of 30‰, 10‰ and in freshwater.
A-type : △ ; Hinta Pond, □ ; Kamitomita Pond, ◇ ; Marutazawa Pond, ○ ; Natori River.
B-type : ● ; Hirose River.

of 23‰. In the medium with the salinity of 30‰, however, the survival rate ranged from 13.3% to 43.7% for the A-type, and from 70.0% to 90.0% for the B-type. Survival rates at 30‰ were clearly lower in the A-type than in the B-type.

Changes in the survival rate during the 20 days in the 30‰ medium are shown in Fig. 1. Survival rapidly declined in the population from Hinata Pond, and gradually in the population from Kamitomita Pond, Marutazawa Pond and

TABLE 2. *Mean and range in percent survival to first postlarvae just after metamorphosis in* Palaemon paucidens *cultured at three different salinities*

Type Locality	Salinity level 30‰	 10‰	 Freshwater
A-type			
Hinata Pond	36.1% (12) [3.3-82.1]	93.2% (8) [85.7-96.6]	60.1% (18) [10.0-26.6]
Kamitomita Pond	67.9% (1)	84.4% (7) [64.0-96.6]	68.1% (2) [58.8-77.3]
Marutazawa Pond	79.5% (16) [37.9-100]	91.3% (18) [37.9-100]	67.9% (16) [16.7-100]
Natori River	58.5% (3) [35.7-85.7]	75.9% (7) [43.5-92.9]	57.9% (5) [16.7-93.1]
mean	60.5%	86.2%	63.5%
B-type			
Hirose River	27.8% (11) [6.7-78.3]	52.6% (14) [3.3-96.2]	0% (11)

Number in parenthesis represents the number of beakers examined.
Range in % survival is given in [ ].

the Natori River, all of which belong to the A-type. On the other hand, in the populations of B-type individuals from the Hirose River and the Kuji River, survival did not decline very much during 20 days. These results indicated that the salinity tolerance of the adult shrimp differed between types, with much higher salinity tolerance in the B-type than in the A-type.

Salinity requirement in larval instars : The changes average survival rate of both A- and B-types during the larval developmental instars are shown in Fig. 2. In type A the survival of larvae at 30‰ declined to about 40%-90% by the third instar, and then there was little further mortality kept until the last molting at metamorphosis to the first instar of postlarva. In the population from the Hirose River which belongs to the B-type, the mean survival declined lenearly to about 30% during the larval stage. After the last molting in larval stage to the first instar of postlarva, the mean survival rate in the population from the Hirose River was 27.8%. Clear differences between the two types were not observed at 30‰.

In the culture medium with a salinity of 10‰, which is used normally to culture larvae of *P. paucidens*, the mean survival declined slightly, to about 80%-90%, in populations of the A-type. Survival declined slightly in the B-type, but not as much as in the A-type. The difference between survival in the two types was the same as in previous reports (3-5).

In the freshwater medium, mean survival of the A-type populations declined to about 60%-70% during the larval developmental instars, while in the B-type population from the Hirose River, survival declined rapidly and all individuals died by the third molt.

The mean survival to the first instar postlarval stage, just after metamorphosis was compared among populations of both types at the three salinities, as shown in Table 2. At a salinity of 30‰, the mean survival ranged from 27.8% (B-type, the Hirose River) to 79.5% (A-type, Marutazawa Pond). Although the survival rate tended to be high in the A-type populations, the variance was also high among populations of the A-type. The survival rate of the postlarvae cultured at a salinity of 10‰ ranged from 75.9% to 93.2% in the A-type, while it was 52.6% in the B-type. The survival rate of postlarvae reared in freshwater ranged from 57.9% to 68.1% in the A-type, but all larvae of the B-type died by the third instar.

Length of larval stage: The development time of A-type larvae to metamorphosis ranged from 9 to 24 days at a salinity of 30‰, 8 to 22 days at a salinity of 10‰, and 9 to 20 days in the freshwater medium. There were no clear differences in development time among A-type larvae reared at different salinities. The period of larval development in the B-type ranged from 17 to 58 days at a salinity of 30‰ and 14 to 40 days at a salinity of 10‰, also indicating no clear differences in development time at the different salinities. In freshwater, the time for larval development could not be measured because no larvae survived until metamorphosis.

Body and carapace length: Mean body and carapace length just after metamorphosis in the first instar of postlarvae of the A-type ranged from 7.5 to 7.8 and 1.9 mm at a salinity of 30‰, 7.5 to 8.3 and 1.9 to 2.0 mm at a salinity of 10‰, and 7.3 to 7.7 and 1.8 to 1.9 mm in freshwater medium, respectively. There were no clear differences in the A-type among larvae reared at the different salinities. In the B-type, the mean body and carapace length was 8.4 and 2.0 mm at a salinity of 30‰ and 8.0 and 1.9 mm at a salinity of 10‰, also indicating no clear difference. In freshwater, the mean body and carapace length at the first instar of postlarvae could not be obtained because no larvae survived until metamorphosis.

Discussion

Salinity, as well as temperature, is one of the most important factors causing the restricted distribution of species. Although *Palaemon paucidens* inhabits upstream and downstream areas of rivers, lakes and ponds, these areas do not offer equivalent environments, especially in regard to salinity. In the rivers, especially the downstream sections, the individual shrimp may have a chance of being carried into an estuary. The difference in the salinity tolerance of adults of the two types indicate that the electrophoretic types may reflect differentiation at the physiological level. The A-type inhabits the upstream sections of rivers, lakes and ponds and the B-type is only found downstream. The capacity of both types for survival at a salinity of 23‰ may reflect the ancestral characteristics. The reason why most of freshwater shrimp including *P. paucidens* is considered to evolved from seawater areas (8).

In the case of freshwater shrimp, a certain level of salinity is required for successful early life (7-12). Ogasawara *et al.* (7) reported that all the zoeas of *Macrobrachium nipponense* reared in freshwater died in early larval stages. Mashiko (8) also reported that high or low salinity induced low survival in *P. paucidens* which had been collected from the estuary of the Sagami River (Kanagawa Pref.), and that an adequate concentration of seawater is necessary for their early life. The results of the present study, in which a requirement for salinity was revealed in the larvae of the B-type, elucidate the earlier studies (7-12). Individual larvae of the B-type could possibly migrate to and grow in brackish water. However, the A-type which has a wider distribution in different types of inland waters is not dependent on brackish water for its life cycle. The study indicated that the two types of *P. paucidens* are adapted to their respective habitats.

Except for *P. paucidens*, species of *Palaemon* inhabit coastal waters around Japan. Mashiko (8) suggested that the inferred evolutionary change from saline to freshwater habitats has occurred in *Palaemon paucidens* based upon its larval physiological requirement for saline water. In the previous report (2), the degree of genetic differentiation among populations of the A-type from different localities was much larger than in the B-type. A higher degree of genetic differentiation among localities could be explained by the longer evolutionary isolation of A-type populations from each other. In accord with suggestions from previous work (2, 7) and based on the results of present study, it is likely that the A-type radiated into freshwater areas before the B-type.

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